American Journal of Hematology 53:228–233 (1996)

Molecular Characterization of β-Thalassemia Mutations in Guadeloupe

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In order to perform genetic counselling and prenatal diagnosis of Hb-S-β-thalassemia disease and β-thalassemia, we have delineated the spectrum of β-thalassemia alleles in the Guadeloupean population. A sample of 63 unrelated families was analyzed including 70 β-thalassemia carriers, 52 Hb-S-β-thalassemia, and 8 patients with different β-thalassemic hemoglobinopathies. Among the eleven mutations identified, four of them [−29 (A → G), IVS-I-5 (G → A), IVS-II-1 (G → A), and IVS-I-5 (G → C)] account for 77.6% of the β-thalassemia chromosomes present in the studied families. The seven other variants, CD 24 (T → A), IVS-I-2 (T → C), Poly A (T → C), −88 (C → T), IVS- II-849 (A → G), Hb E, and Hb Lepore are less frequent. As a result, Hb S-β$^+$-thalassemia type 1 (low Hb A values: 5–15%) together with Hb S-β^0-thalassemia phenotypes are as frequent as Hb S-β$^+$-thalassemia type 2 (high Hb A values: 20–30%) in the Guadeloupean population. Patients with Hb S-β$^+$-thalassemia type 2 have milder hematological manifestations of the disease compared to patients with Hb S-β^0-thalassemia and Hb S-β$^+$-thalassemia type 1. This first report on the type and nature of β-thalassemia mutations in Guadeloupe shows that prenatal diagnosis of Hb S-β-thalassemia and β-thalassemia should be feasible by direct detection of point mutation in most cases. © 1996 Wiley-Liss, Inc.

Key words: β-thalassemia mutations, β-thalassemia haplotypes, Hb S-β-thalassemia

INTRODUCTION

Sickle cell disease (SCD) is a generic term for a family of hemoglobin disorders having in common inheritance of a sickle β-globin gene. Sickle cell anemia is the homozygote state (SS) whereas other commonly encountered SCDs result from the coinheritance of β^S with either a β-thal gene or β-chain structural variant such as β^C [1]. As in many other populations of African origins, SCD constitutes a major public health problem in Guadeloupe. Guadeloupe is a French-speaking island (1,709 km^2) in the Caribbean with a population (413,000) of predominant admixture of populations of West African, Asian Indian, and Caucasian ancestry. The most common β-globin chain abnormalities in this population, β^S and β^C, occur with gene frequencies of 0.044 and 0.013, respectively, whereas the frequency of β-thalassemia (β-thal) gene has been estimated to be 0.005. A preventive program aimed to control SCD in this population, based on newborn screening, medical care, counselling, and prenatal diagnosis, was started in 1990.

Among SCD syndromes observed in the Guadeloupean population, the occurrence of Hb S-β-thal is significant, however, lower than that of SS and SC genotypes. Three different types of Hb S-β-thal phenotypes are distinguished: Hb S-β^0-thal without Hb A, Hb S-β$^+$-thal type 1 with 5 to 15% of Hb A, and Hb S-β$^+$-thal type 2 with 20 to 30% of Hb A [1]. Because of the high morbidity and mortality of Hb S-β-thal as well as β-thal disease, prenatal diagnosis has been an important option for couples in which both members are carriers of a β-thal gene and/or a β^S-globin gene. A prerequisite for such a program is to establish the spectrum of β-thal mutations in the target population. Indeed, in contrast to β^S- and β^C-globin genes, over 160 different mutations causing β-thalassemia or thalassemic syndrome (e.g., Hb E, Hb Lepore) have been identified so far [2]. Most of these molecular

Received for publication January 19, 1996; accepted June 18, 1996.

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defects are single base substitutions or small insertions or deletions within or flanking the β-globin gene. In general, each population has a variety of β-thal mutations, consisting of a very few common ones and a variable number of rare ones [3].

This is the first report on characterization of β-thal alleles present in the Guadeloupean population, the spectrum and distribution of β-thal mutations as well as haplotype/mutation associations among the Guadeloupean was delineated. The relationship between hematological phenotype and the type of β-thal mutation in patients with Hb S-β-thal is also discussed.

METHODS AND SUBJECTS

Sixty-three unrelated β-thal carrier families were studied. One hundred and thirty members of these families, carriers of at least one β-thal chromosome, attended the comprehensive sickle cell center either for follow-up care of their hemoglobinopathies (Hb S-β^+-thal:36; Hb S-β^0-thal:16; β-thal-β-thal:2; Hb S-Hb E:1; Hb S-Hb Lepore:1; Hb Hope-β-thal:1) or addressed for diagnosis in a carrier screening program (Hb A-β-thal:70; Hb C-β-thal:2; hereditary persistence of fetal hemoglobin [HPFH]-β-thal:1). A total of 132 β-thal chromosomes was studied which included two patients with β-thal disease.

Peripheral blood (5–10 ml) was collected on EDTA as anticoagulant. Informed consent was obtained from patients or parents with affected children. The diagnosis of β-thal trait was based on the association of microcytosis, hypochromia, and elevated level of Hb A2 and hence the eventual presence of silent β-thal was not looked for. Hematological data were obtained with an automated cell counter and with routine hematological tests. Hb in red cell lysates were studied by isoelectrofocusing, citrate agar electrophoresis at pH 6.0, and by cation exchange high performance liquid chromatography (HPLC) [4]. Hb A2 quantification was established by DEAE microcolumn chromatography and Hb F level estimation by HPLC [5] was also carried out.

DNA was isolated from peripheral leukocytes as described by Poncz et al. [6]. Amplification of β-globin gene regions by polymerase chain reaction (PCR) was carried out as previously described [7,8]. Different β-thal mutations were identified by dot-blot analysis of amplified DNA, and hybridization with 32P-labeled allele specific oligonucleotides. Some of the β-thal mutations were identified by direct DNA sequencing of PCR products [9]. Haplotype analysis was carried out for the most common β-thal alleles. The polymorphic restriction sites *Hinc*II-5′ε, *Xmn*I-5′^{G}γ, *Hind*III-^{G}γ, *Hind*III-^{A}γ, *Hinc*II-ψβ, *Hinc*II-3′ψβ, *Hinf*I-5′β, and *Aval*I-β were explored as have been described earlier [10–12].

The results are expressed as means ± SD. Means were compared using analysis of variance (ANOVA) or Student's *t*-test. Proportions were compared using χ^2 test with correction for continuity when necessary.

RESULTS

In the 63 unrelated families studied, 12 of them carried a β^0-thal mutation whereas the 51 others a β^+-thal mutation. Characterization of β-thal alleles in the study sample had revealed a molecular heterogeneity with 11 mutations identified.

Table I lists the different β-thal alleles observed and the population in which these mutations had been previously found [2,13]. Four prevalent mutations, with frequencies higher than 10%, account for 77.6% of the molecular defects detected in these families. The mild A → G mutation at position −29 within the TATA box of the β-globin promoter is by far the most common defect (41.2%), followed by the RNA processing mutations, namely IVS-I-5 (G → A) (14.2%), IVS-II-1 (G → A) (11.1%), and IVS-I-5 (G → C) (11.1%). The seven other alleles appear less frequently, namely CD24 (T → A), IVS-I-2 (T → C), Poly A (T → C), IVS-II-849 (A → G), −88 (C → T), Hb E, and Hb Lepore.

We carried out haplotype analysis for the four most frequent β-thal mutations (Table II). Each thalassemic mutation was found to be associated with a specific haplotype, except for the −29A → G mutation. This mutation was found in association with 7 different β-globin cluster haplotypes.

Among the patients studied, only two of them were homozygous β-thalassemia. A 27-year-old woman was homozygote for the −29 A → G mutation. She was transfusion free, her steady-state hematologic indices (Hb 10.5 g/dl, MCV 69.9 fl, Hb A 30%, Hb F 69.9%, Hb A2 4.3%) and clinical manifestations were consistent with β-thal intermedia. The second patient, an 8-month-old boy, was compound heterozygote for the Poly A (T → C) and IVS-I-5 (G → C) mutations. This child with severe anemia had received multiple transfusions. Hematologic and Hb data obtained away from transfusion (Hb 5.1 g/dl, MCV 67 fl, Hb A 8%, Hb F 90%, Hb A2 1.7%) confirmed the clinical diagnosis of β-thal major.

Two patients compound heterozygotes for Hb S-Hb E and Hb S-Hb Lepore were also detected. These two abnormal hemoglobin variants are known to be associated with a β^+-thal phenotype. Two other patients were found to be compound heterozygote for Hb Hope-β^+-thal (Poly A (T → C)) and HPFH-β^+-thal (−29 (A → G)).

Analysis of blood samples from the β-thal carriers was consistent with the typical manifestation of β-thal trait; all had a marked microcytosis (MCV 68.7 ± 6.1 fl) with hypochromia (MCH 21.2 ± 1.8 pg), a slight anemia (Hb 11.55 ± 1.5 g/dl), an elevated Hb A2 level (4.7 ± 0.8%), and a moderately elevated Hb F (3.9 ± 4.8%). The hematological findings of these β-thal heterozygotes were eval-

TABLE I. Prevalence of the 11 Different β-Thalassemia Mutations Observed in Guadeloupe*

Mutations	Type	No of families (chromosomes)	%	Origin of previously described mutations
-29 A → G	β^+	26 (51)	41.2 [38.6]	African American, Chinese
IVS-I-5 G → A	β^+	9 (12)	14.2 [9.1]	Algerian
IVS-II-1 G → A	β^0	7 (19)	11.1 [14.4]	African American, Mediterranean
IVS-I-5 G → C	β^+	7 (15)	11.1 [11.4]	Asian Indian, Chinese
IVS-I-2 T → C	β^0	3 (5)	4.8 [3.8]	African American, Algerian
CD24 T → A	β^+	3 (4)	4.8 [3]	African American, Japanese
Poly A T → C	β^+	2 (9)	3.2 [6.8]	African American
IVS-II-849 A → G	β^0	2 (7)	3.2 [5.3]	African American
-88 C → T	β^+	2 (4)	3.2 [3]	African American, Asian Indian
Hb Lepore	β^+	1 (3)	1.6 [2.3]	African American
Hb E	β^+	1 (3)	1.6 [2.3]	S.E. Asian
Total		63 (132)	100%	

*The numbers in parentheses refer to the number of β-thal chromosomes. The numbers in brackets refer to the percentage of β-thal chromosomes.

TABLE II. β-Thalassemia Mutation and Haplotype Association in the Guadeloupean Population

		Polymorphic restriction sites							
Mutations	n[a]	*Hinc*II 5′-ε	*Xmn*I 3′-ε	*Hind*III $^G\gamma$	*Hind*III $^A\gamma$	*Hinc*II Ψβ	*Hinc*II 3′-Ψβ	*Hinf*I 5′-β	*Ava*II β
-29 A → G	8(7)	−	−	+	−	+	+	+	+
	4	−	−	+	−	−	+	+	+
	2	−	−	−	−	−	+	−	+
	2	+	−	−	−	−	−	+	+
	1	−	−	+	−	+	−	+	+
	1	−	+	+	−	+	+	+	+
	1	−	+	−	−	−	−	+	+
IVS-I-5 G → A	7(2)	+	−	−	−	−	−	+	+
IVS-I-5 G → C	7(0)	+	−	−	−	−	−	+	−
IVS-II-1 G → A	5(2)	−	−	−	−	−	+	+	+

[a]Numbers of unrelated families carrying the β-thal haplotype. Numbers in parentheses represent numbers of unrelated families for which a definite assignment of haplotypes was not possible owing to heterozygosity for a particular RFLP and not availability of family members.

uated according to the type of molecular defect: β^+-thal 51 [−29 (A → G) 26, IVS-I-5 (G → C) 11, IVS-I-5 (G → A) 7, Poly A (T → C) 5, −88 (C → T), 1, CD24 (T → A) 1], and β^0-thal 15 [IVS-II-1 (G → A) 11, IVS-II-849 (A → G) 3, IVS-I-2 (T → C) 1]. It was noted that age, MCH, MCHC, Hb A2, and Hb values were comparable between the two groups. The Hb F values in patients with β^0-thal alleles (7.5 ± 8%) were significantly higher than those with β^+-thal mutations (2.9 ± 2.7%) ($P < 10^{-3}$). Moreover, patients with β^0-thal alleles had much more pronounced microcytosis (64.7 ± 4.6 fl) than those with β^+-thal defects (69.6 ± 6.0 fl) ($P < 1.5\ 10^{-2}$).

Distribution of Hb S-β-thal individuals according to the level of Hb A in the peripheral blood was as follows: 16 patients with Hb S-β^0-thal [IVS-II-1 (G → A) 8; IVS-I-2 (T → C) 4; IVS-II-849 (A → G) 4], 8 patients with Hb S-β^+-thal type 1 [IVS-1-5 (g → A) 5; IVS-1-5 (G → C) 3], and 28 patients with Hb S-β^+-thal type 2 [−29 (A → G) 21; CD24 (T → A) 3; −88 (C → T) 2; Poly A (T → C) 2]. These 52 patients belonged to 40 unrelated families. Haplotyping of the β^S chromosome was performed for 50 of them. Thirty-nine of them had haplotype 19 or the Benin type, three with haplotype 3, and three with the Senegal type and haplotype 20 or the Bantu type. Five patients had atypical haplotypes.

Hematological and hemoglobin data for these patients are shown in Table III. Patients younger than 3 years old were excluded from the analysis: 1 patient with Hb S-β^+-thal type 1 [IVS-I-5 (G → A)], 7 patients with Hb S-β^+-thal type 2 [−29 (A → G) 5; −88 (C → T) 1; Poly A (T → C) 1], and 1 with Hb S-β^0-thal [IVS-I-2 (T → C)]. The patients with the Hb S-β^+-thal type 2 with Hb A levels of 17.9 ± 5.1% had milder anemia ($P < 10^{-4}$), hypochromia ($P < 10^{-2}$) as well as less marked microcytosis ($P < 2\ 10^{-2}$) as compared to the Hb S-β_+-thal type 1 and Hb S-β^0-thal. Two Hb C-β^+-thal patients,

TABLE III. Hematological Features of Different Types of Sickle Cell β-Thalassemia in Guadeloupe*

	Hb S-β^+-thal type 1	Hb S-β^+-thal type 2	Hb S-β^0-thal
n	7	21	15
Age	9.7 ± 8.8	18.5 ± 13.7	22.0 ± 11.2
Hb (g/dl)	8.7 ± 1.0	11.5 ± 1.6	8.7 ± 1.4
MCV (fl)	67.4 ± 5.6	74.0 ± 4.3	72.9 ± 4.7
MCH (pg)	20.8 ± 1.8	22.9 ± 1.4	23.4 ± 2.3
MCHC (g/dl)	30.8 ± 0.6	30.8 ± 1.4	32.0 ± 2.5
Hb A (%)	6.0 ± 4.5	17.9 ± 5.1	0
Hb A2 (%)	4.6 ± 0.7	4.1 ± 0.9	4.1 ± 0.8
Hb F (%)	14.6 ± 16.1	10.9 ± 7.1	13.5 ± 9.5

*-Hb S-thal$^+$-thal type 1: IVS-I-5 (G → A) 4, IVS-I-5 (G → C) 3. -Hb S-β^+-thal type 2: -29 (A → G) 16, CD24 (T → A) 3, -88 (C → T) 1, Poly A (T → C) 1. -Hb S-β^0-thal: IVS-II-1 (G→A) 8, IVS-I-2 (T→C) 3, IVS-II-849 (A → G) 4.

TABLE IV. Comparison of Relative Frequencies of β-Thalassemia Mutation in Guadeloupeans and American Blacks

Mutation	Guadeloupean population (%)	American Blacks (%)
-29 (A → G)	41.2	60.1
IVS-I-5 (G → A)	14.2	0
IVS-II-1 (G → A)	11.1	0.8
IVS-I-5 (G → C)	11.1	0
IVS-I-2 (T → C)	4.8	0.8
CD24 (T → A)	4.8	2.3
Poly A (T → C)	3.2	0.8
IVS-II-849 (A → G)	3.2	2.3
-88 (C → T)	3.2	21.1
Hb Lepore	1.6	0.8
Hb E	1.6	0[a]
Others	0	11
Total	100	100

[a]Hb E has been detected in African Americans by other groups [20, 21].

one with −29 (A → G) and the other with −88 (C → T) exhibited hematological features similar to these of Hb S-β^+-thal type 2 except for MCV and lower values. The anemia in patients with Hb S-β^+-thal type 1 and Hb S-β^0-thal was similar although microcytosis (67.4 ± 5.6 vs. 72.9 ± 4.7 fl) and hypochromia (20.8 ± 1.8 vs. 23.4 ± 2.3 pg) were more pronounced in patients of the first group. No statistical difference in Hb A2 level was observed. The Hb F values were quite variable in these three groups, ranging from 1 to 25% for Hb S-β^+-thal type 1, 3 to 24.5% for Hb S-β^+-thal type 2, and 2.4 to 32% for Hb S-β^0-thal.

DISCUSSION

Newborn screening, genetic counseling, peri and prenatal diagnosis have proven to be efficient in the control and management of hemoglobinopathies [14]. Since nature and prevalence of β-thal mutations are different between population groups, we explored the spectrum of β-thalassemia mutations present in the Guadeloupean population in order to implement a thalassemia control program in this region.

The Guadeloupean population is the result of recent admixture between different ethnic groups. Among them, the West African group is believed to be numerically the largest one. Starting during the sixteenth century and ending in the middle of the eighteenth, about 300,000 slaves were imported into Guadeloupe [15]. Angola, the Bight of Benin, the Windward Coast, and the Gold Coast contributed most of these slaves. From 1854 until 1885, 40,000 people were imported from India, most of them (30,000) from Southern India, the others from the North part of the country [16]. The smallest Caucasian group is composed essentially of peoples from metropolitan France, Syria and Lebanon, these latter Mediterraneans arriving during the middle of this century as traders.

Since from epidemiological data β-thal gene frequency in Guadeloupe is estimated to be 0.005, the 132 chromosomes characterized in the present study correspond approximately to 5% of β-thal alleles in the Guadeloupean population. Eleven different β-thal mutations have been identified in this sample. In Table IV, we compared the frequency of β-thal mutations detected in this study with a closely related ethnic group, i.e., African Americans [17]. The −29 (A → G) mutation is by far the most common allele in these two groups, albeit at different frequencies (41.2 vs. 60.1%). Further discrepancies for the −88 (C → T) (3.2 vs. 21.1%) and IVS-II-1 (G → A) mutations (11.1 vs. 0.8%) are also observed. On the other hand, the much less common four β-thal variants, namely, CD 24 (T → A), IVS-I-2 (T → C), Poly A (T → C), and Hb Lepore are present in both groups. Although Hb E has not been reported by Gonzalez-Redondo et al. [17], this β-globin variant has been described by other groups in Black Americans [18,19] and therefore could be considered a rare allele. Absence of IVS-I-5 (G → C) and IVS-I-5 (G → A) mutations in African Americans contrasted with their presence in our population and suggested that these two molecular defects reached Guadeloupe by gene-flow from other areas than West Africa.

The association between haplotype and β-thal mutation may provide much more precise information regarding the gene-flow on this island [20]. The substitution (G → C) at IVS-I position 5 is interesting because it has been found in the Chinese [21], Asian Indian [22], and Mediterranean population [23]. This mutation lies on different haplotypes in each population suggesting that it had arisen independently in each. In Guadeloupe, the linkage of this mutation to the haplotype (+ − − − − − + −) strongly suggests an Asian Indian origin. A similar observation can be made for the IVS-II-1 (G → A) mutation which

is prevalent in Mediterranean populations [24] and also detected in African American [17,25]. The strong association between this molecular defect and the haplotype (− − − − − + + +) supports the hypothesis of a West African origin [26,10]. All the seven unrelated individuals with the IVS-I-5 (G → A) mutation available for DNA studies had identical (+ − − − − − + +) haplotype. This mutation is a rare allele in Mediterraneans [24]. The haplotype/mutation for this substitution has been described [27] and is similar to the haplotype characterized in this study. Therefore, given the historical records, it is conceivable that this mutation had reached Guadeloupe by gene-flow from Mediterranean area. The promoter −29 (A → G) mutation detected in our sample is very likely to be of West African input. Indeed, among the seven different haplotypes associated with this substitution, four of them have been previously observed in African Americans [28,7]. Novel association of this mutation to three additional haplotypes [(− − + − − + + +), (− + − − − − + +), and (− − + − + − + +)] have been detected. There are three possible explanations for this remarkable degree of heterogeneity of haplotype distribution observed only for this β-thal mutation: (1) The promoter −29 (A → G) mutation arose more than once in West Africa. (2) The −29 (A → G) mutation occurred only once, and the new chromosome haplotypes were generated by recombination events. (3) The diversity results from a combination of multiple mutations and recombination events. Characterization of the restriction sites 3′ to the thalassemic β-globin gene as well as chromosome haplotypes of the normal Guadeloupean population will be necessary to discriminate between these different hypotheses.

As in other SCD syndromes, variability of clinical expression in sickle cell β-thalassemia is not fully understood [1]. However, one of the determinants of Hb S-β-thal clinical severity is the extent of impairment of Hb A production; β^0-thal genes do not direct the production of any Hb A and often have greater clinical impact than β^+-thal genes, which has some residual production. Among the patients studied with Hb S-β-thal disease, more than half of them belonged to the Hb S-β^+-thal type 2 group (53.8%) followed by the Hb S-β^0-thal (30.8%) and Hb S-β^+-thal type 1 (15.4%). As has been shown by others [7,29,30], patients with Hb S-β^+-thal type 2 had less severe hematological manifestations of the disease as compared to those with Hb S-β^0-thal and Hb S-β^+-thal type 1 (Table III). While the degree of anemia was equivalent between patients of the two latter phenotypes, paradoxically microcytosis and hypochromia were much more pronounced in Hb S-β^+-thal type 1. Further investigations such as α-globin gene status and iron deficiency are necessary to explain these discrepancies. The clinical expression of the Hb S-β^+-thal disease is also related to the Hb F level [29,30]. Wide variation in the range of Hb F level observed in all three groups could not be related to the C → T substitution at position −158 of the $^G\gamma$-globin gene since the *Xmn*I polymorphic site was absent in most of these β^S- and β-thal haplotypes. Since parental Hb F values were not available in some instances, no definite conclusion could be drawn concerning the coinheritance of a Swiss type heterocellular HPFH.

In conclusion, a wide spectrum of β-thal mutations was observed in Guadeloupe, with four of them accounting for more than 70% of the β-thal mutation in this population. Haplotype association studies bring arguments for gene-flow from West Africa, India, and Mediterranean areas. Sickle cell β-thalassemia in Guadeloupean population exhibits specific features. Interestingly, while the Hb S-β^+-thal type 2 is predominant in Jamaicans and African Americans and the Hb S-β^+-thal type 1 is predominant in Mediterraneans, all the sickle cell β-thalassemia types, namely, Hb S-β^0-thal, Hb S- β^+-thal type 1 and type 2, are significantly represented in the Guadeloupean population, strongly suggesting that the population admixture is much more pronounced in this island than the other cited regions. As a practical means of control of β-thalassemia, this study has paved the way for mutation oriented molecular diagnosis by reverse dot-blot technique [31].

ACKNOWLEDGMENTS

We thank the personnel of the pediatric services of the Centre Hospitalier Universitaire of Pointe-à-Pitre, the Centre Intégré de la Drepanocytose, Alexis Elbaz, Rajagopal Krishnamoorthy, and Josué Feingold for helpful discussion as well as M. Cohen-Solal for constant support during this study. This work was supported by grants from INSERM, the Conseil Général de la Guadeloupe, and CORDET. We dedicate this paper to our beloved director Guy Mérault who initiated and conducted this study.

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